

SUPPLEMENTAL MATERIAL

Phosphoribosyl-diphosphate (PRPP): Biosynthesis, Enzymology, Utilization, and Metabolic Significance

Bjarne Hove-Jensen,^{a,b} Kasper R. Andersen,^b Mogens Kilstrup,^a Jan Martinussen,^a Robert L. Switzer,^c Martin Willemoes^d

DTU Systems Biology, Technical University of Denmark, Kongens Lyngby, Denmark^a; Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark^b; Department of Biochemistry, University of Illinois at Urbana-Champaign, USA^c; Department of Biology, University of Copenhagen, Copenhagen, Denmark^d

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REFERENCES

TABLE S1 Comparison of amino acid residues of the active site of *B. subtilis* PRPP synthase with amino acid residues of PRPP synthase of other organisms^a

<i>B. subtilis</i>	<i>E. coli</i>	<i>M. tuberculosis</i>	<i>S. oleracea</i> isozyme 4	<i>M. jannaschii</i>	<i>T. volcanium</i>	<i>S. solfataricus</i>
Binding the adenyl moiety of ATP (<i>B. subtilis</i> PRPP synthase, subunit D) ^b						
F40	F35	F43	F41	F32	F32	F32
D42	D37	N45	D43	D34	D34	D34
E44	E39	E47	N47	E36	D36	E36
V45	V40	I48	L48	I37	L37	S37
Binding the adenyl moiety of ATP (<i>B. subtilis</i> PRPP synthase, subunit A)						
Y99	Y94	Y102	T100	Y90	Y89	Y91
T113	T119	S116	T114	S104	S103	S105
ATP phosphate chain binding, RFL, PP loop and CFL (<i>B. subtilis</i> PRPP synthase, subunit A) ^c						
R101	R96	R104	S102	R92	R91	R93
Q102	Q97	Q105	F103	Q93	Q92	Q94
D103	D98	D106	E104	D94	H93	D95
R104	D99	D107	R105	K95	Q94	R96
H135 ^d	H131	H138	H141	H125	H124	H127
D174 ^d	D170	D178	D183	D163	D161	D165
G176	G172	G180	G185	G165	G163	G167
G177	G173	R181	A186	A166	G164	A168
K197	K194	K202	K204	K186	K184	K188
R199	R196	R204	R206	K188	R186	R190
N203	N200	N210	D209	T192	R190	T194
Ribose 5-phosphate binding (<i>B. subtilis</i> PRPP synthase, subunit A)						
D223	D220	D230	D229	D212	D210	D216
D224	D221	D231	D230	D213	D211	D217
D227	D224	D234	Q233	S216	S214	S220
T228	T225	T235	S234	T217	T215	T221
A229	G226	G236	G235	G218	G216	G222
T231	T228	T238	T237	T220	T218	T224
I232	L229	I239	L238	M221	I219	I225

^a The annotated amino acid residues of the active site of *B. subtilis* PRPP synthase are listed at the left-hand column according to previously published three-dimensional structures (1-3). The *B. subtilis* PRPP synthase amino acid sequence was aligned pairwise with the amino acid sequences of PRPP synthases of *E. coli*, *M. tuberculosis*; *S. oleracea* isozyme 4; *M. jannaschii*; *T. volcanium* and *S. solfataricus*, and the amino acid residues of these enzymes at similar positions to those of the *B. subtilis* enzyme are listed in the various columns. Residues that are identical or conserved relative to the *B. subtilis* enzyme are typed in bold, whereas non-conserved residues are typed in red.

^b The amino acid residues are organized according to their function in substrate binding. The subunits harboring the respective amino acid residues are given in parenthesis, i.e. subunit A or subunit D (Fig. 4B). Subunits C and F or E and B share identical functions (Fig. 4C).

^c RFL, regulatory flexible loop; CFL, catalytic flexible loop.

^d His135 and Asp174 are also involved in the binding of Mg²⁺.

TABLE S2 Comparison of amino acid residues of the allosteric site of *B. subtilis* PRPP synthase with amino acid residues of PRPP synthase of other organisms^a

<i>B. subtilis</i>	<i>E. coli</i>	<i>M. tuberculosis</i>	<i>S. oleracea</i> isozyme 4	<i>M. jannaschii</i>	<i>T. volcanium</i>	<i>S. solfataricus</i>
Hydrophobic pocket (<i>B. subtilis</i> PRPP synthase, subunit B) ^b						
L134	L130	L137	I140 ^c	P124	H124	P126
I139	I135	I142	E145	I129	L129	E131
Q140	Q136	Q143	R146	K130	S130	L132
D148	D144	D151	L154	I138	D138	L139
H149	N145	H152	P155	Y139	L139	K140
Regulatory flexible loop (<i>B. subtilis</i> PRPP synthase, subunit A)						
K105	R100	K108	E109	K96	R95	R97
A106	V101	H109	G110	F97	Y96	F98
R107	R102	R110	D111	N98	K97	K99
S108	S103	G111	V112	P99	N98	D100
R109	R105	R112	A113	G100	G99	G101
Binding of the β-phosphate and the adenyl moiety of ADP (<i>B. subtilis</i> PRPP synthase, subunit D)						
S52	N47	S55	D55	E44	D44	S44
R54	R49	R57	R57	N46	T46	R46
A85	A80	G88	A89	E76	T77	L77
S86	S81	S89	S90	G77	K78	G78
Binding of the β-phosphate and the adenyl moiety of ADP (<i>B. subtilis</i> PRPP synthase, subunit B)						
S310	S308	S317	I312	NF ^d	NF	NF
V311	I309	V318	I312	NF	NF	NF
S312	S310	T319	D314	NF	NF	NF
Y313	A311	G320	A315	NF	NF	NF
L314	M312	L321	L316	NF	NF	NF
F315	F313	F322	Q317	NF	NF	NF

^a Amino acid residues that are identical or conserved relative to the *B. subtilis* enzyme are shown in bold, whereas non-conserved residues are shown in red. The annotated amino acid residues of the allosteric site of *B. subtilis* PRPP synthase are listed at the left-hand column according to the previously published three-dimensional structure (2). Amino acid residues at similar positions in PRPP synthases of *E. coli*, *M. tuberculosis*, *S. oleracea* isozyme 4, *M. jannaschii*, *T. volcanium* and *S. solfataricus* are listed in the other columns. Color code of amino acid residues is that of Table S1.

^b The subunits harboring the respective amino acid residues are given in parenthesis, i.e. subunits A, B and D (Fig. 4B). Similar contributions of amino acid residues are offered by subunits D, C and F, or by subunits F, E and B (Fig. 4C).

^c The I140 of *S. oleracea* isozyme 4 is indicated as non-homologous, as a leucine to isoleucine alteration at this position in human PRPP synthase isozyme 1 and *B. amyloliquefaciens* PRPP synthase (L129I and L135I, respectively) resulted in loss of allosteric inhibition (4, 5).

^d NF, not found. The amino acid sequences of PRPP synthases from *M. jannaschii*, *T. volcanium* and *S. solfataricus* are 284, 286 and 291 amino acids long, respectively.

TABLE S3 Comparison of amino acid residues of the active site of *B. subtilis* PRPP synthase with amino acid residues of classI and archaeal PRPP synthases^a

<i>B. subtilis</i>	<i>L. lactis</i>		<i>S. cerevisiae</i>					<i>S. pombe</i>			Human		<i>A. fulgidus</i> ^b	
	<i>prsA</i>	<i>prsB</i>	1	2	3	4	5	1	2	3	I	PAP39	1	2
Binding the adenyl moiety of ATP of <i>B. subtilis</i> PRPP synthase														
F40	H37	F39	F35	Y37	D37	Y37	F37	F35	F35	Y37	F35	E42	F32	Y32
D42	D39	D41	N37	N39	T39	N39	N39	N37	N37	N39	N37	N44	D34	D34
E44	E41	E43	E39	E41	E41	E41	E41	E39	E39	E41	E39	E46	E36	E36
V45	V43	I44	T50	T42	V42	T42	T42	T40	T40	T42	T40	T47	L37	K47
Y99	Y96	Y98	Y94	Y96	Y87	Y98	F208	Y94	Y94	Y96	Y94	Y101	Y80	Y71
T113	T110	T112	T108	T110	T110	T112	P110	A222	I134	T110	S108	V114	S94	P104
ATP phosphate chain binding, regulatory flexible, PP and catalytic flexible loops of <i>B. subtilis</i> PRPP synthase														
R101	R98	R100	K96	R100	R99	R100	A210	K96	R96	R98	R96	K103	R82	R93
Q102	Q99	Q101	Q97	Q101	Q100	Q101	Q211	Q97	Q97	Q99	Q97	Q104	Q83	S94
D103	D100	D103	C98	D102	D101	D102	N212	S98	P98	D100	D98	S105	D84	L95
R104	R101	R104	K99	K103	R102	K103	A213	K99	D99	K101	K99	K106	K85	D96
H135	H132	H135	H130	H134	H133	H134	H244	H130	H154	H132	H130	H136	H115	H126
D174	D174	K175	N176	D173	D173	D174	D285	N169	D195	D174	D171	S177	D150	D163
G176	G176	S177	G178	G175	G175	G176	G287	G171	G197	G176	G173	D179	G152	K165
G177	G177	G178	G179	G176	G176	G177	G288	G172	G198	G177	G174	A180	S153	A166
K197	K197	Y198	K192	K197	K194	K197	K308	T192	K217	K197	K194	G200	K173	K186
R199	R199	D200	R194	R199	R196	R199	R310	R194	R219	R199	R196	H202	R175	R188
N203	N203	N204	L198	N203	N200	N203	K377	S198	E222	N203	N200	E206	T179	E192
Ribose 5-phosphate binding of <i>B. subtilis</i> PRPP synthase														
D223	D223	D223	D325	D223	D222	D223	D397	D309	D242	D22	D220	D256	D199	D211
D224	D224	D224	D326	D224	D223	D224	D398	D310	D243	D224	D221	D257	D200	D212
D227	D227	N227	D329	D227	D226	D227	D401	E313	D246	D227	D224	D260	S202	S205
T228	T228	T228	R330	T228	T227	T228	T402	N314	T247	T228	T225	D261	T204	T206
A229	A229	G229	P331	C229	C228	C229	S403	P315	A248	C229	C226	V262	G205	G207
T231	T231	T231	S333	T231	T230	T231	T405	A317	T250	T231	T228	S264	T207	T219
I232	I232	F232	F334	L232	L231	L232	I406	F318	L251	L232	I229	F265	V208	V220

^a Amino acid residues that are identical or conserved relative to the *B. subtilis* enzyme are shown in bold, whereas non-conserved residues are shown in red. The annotated amino acid residues of the active site of *B. subtilis* PRPP synthase, similar to those of Table S1, are listed at the left-hand column. Accession number or reference for the sequences are X15331 and Y0097 for human PRPP synthase isozyme 1 and 2, respectively (6); human PAP39 (7). Human PAP41 is not shown as only one amino acid of the selected amino acids differ among PAP39 and PAP41 (8); BK006944 (chromosome XI) (*S. cerevisiae* PRPP synthase 1) (9); BK006939 (chromosome V) (*S. cerevisiae* PRPP synthase 2) (10); BK006934 (chromosome VIII) (*S. cerevisiae* PRPP synthase 3) (11); BK006936 (chromosome II) (*S. cerevisiae* PRPP synthase 4) (12); BK006948 (chromosome XV) (*S. cerevisiae* PRPP synthase 5) (13); CU329670 (chromosome I) (*S. pombe* PRPP synthase 1); CU329671 (chromosome 2) (*S. pombe* PRPP synthase 2); CU329672 (chromosome 3) (*S. pombe* PRPP synthase 3) (14); AE000782 (*A. fulgidus* DSM 4304) (15); AM406671 (*L. lactis* subsp. *cremoris* MG1363) (16).

^b *A. fulgidus* 1 is the 271 amino acid putative PRPP synthase (accession AIG98299), whereas *A. fulgidus* 2 is the 284 amino acid putative PRPP synthase (accession AIG97465) (15).

TABLE S4 Complementation of *E. coli* Δprs by *S. pombe* PRS genes and activity of PRPP synthase^a

Plasmid (PRS gene or genes)	Growth		PRPP synthase activity nmol (min \times mg protein) $^{-1}$
	- NAD	+ NAD	
pHO548 (<i>PRS1</i>)	-	+	ND ^b
pHO535 (<i>PRS2</i>)	-	+	ND
pHO547 (<i>PRS3</i>)	-	+	ND
pHO550 (<i>PRS1 PRS2</i>)	-	+	ND
pHO551 (<i>PRS1 PRS3</i>)	+	+	5
pHO552 (<i>PRS2 PRS3</i>)	+	+	4
pHO11 (<i>prs</i> ⁺ <i>E. coli</i>)	+	+	1750 ^c
pBR322 (None)	-	+	ND

^a The host strain was HO773 (Δprs), which requires guanosine, uridine, histidine, tryptophan and NAD (17). Transformed cells were plated on rich medium, which contains guanosine, uridine, histidine, and tryptophan. NAD was supplemented as indicated. Complementation, i.e. synthesis of PRPP synthase results in NAD prototrophy (18). Growth was recorded after 48 h of incubation at 30 °C. -, no growth; +, growth. The plasmids were derived from pBR322 (19). Construction will be described elsewhere. In effect an *S. pombe* PRS coding sequence replaced the *E. coli* *prs* coding sequence of pHO11 (20). PRPP synthase activity was determined as previously described (21).

^b ND, not determined.

^c Value published previously (20)

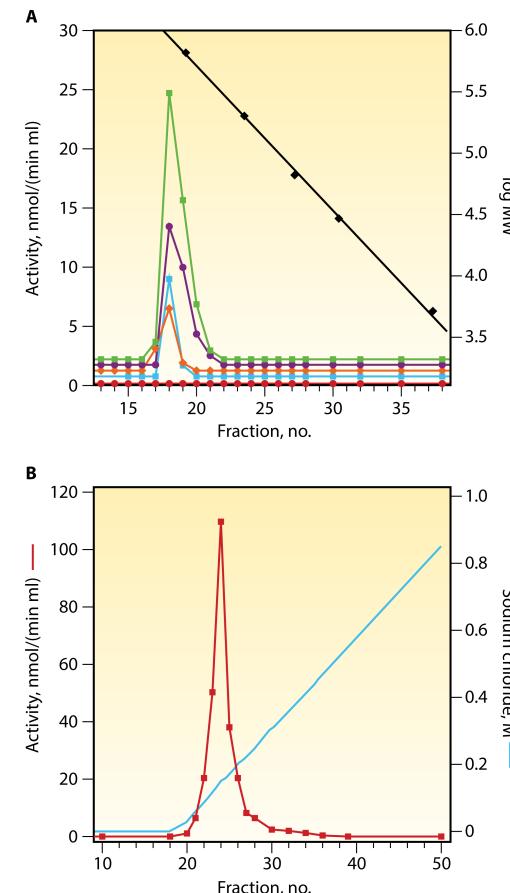


FIG S1 Chromatographic analysis of *S. cerevisiae* PRPP synthase. Stationary phase cultures of YPD-grown *S. cerevisiae* cells (kindly provided by M. Schweizer, Heriot-Watt University, Edinburgh, UK) were harvested by centrifugation, resuspended in 50 mM potassium phosphate buffer (pH 7.6) containing 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 1 mM EGTA, 1 μ g mL $^{-1}$ leupeptin and broken by agitation in the presence of 0.5 μ m glass beads in a Bead Beater (Biospec Products, Bartlesville, OK). (A) Exclusion chromatography. Color code and amount of PRPP synthase activity [determined as previously described (21)] applied to the column: green line, wild-type strain YN94-2, 1.07 μ mol min $^{-1}$; red line, $\Delta prs1$ strain YN96-66, 0.45 μ mol min $^{-1}$; blue line, $\Delta prs2$ strain YN97-7, 0.72 μ mol min $^{-1}$; orange line, $\Delta prs3$ strain YN96-67, 0.75 μ mol min $^{-1}$; purple line, $\Delta prs4$ strain YN97-6, 0.57 μ mol min $^{-1}$. In addition, 0.68 μ mol min $^{-1}$ of PRPP synthase of $\Delta prs5$ strain YN96-69 was applied to the column (elution profile not shown). Black line, calibration of the column. Standards for molecular mass estimation were thyroglobulin (molecular mass 670 kDa), β -amylase (200 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa) and ADP (0.4 kDa). Four of the activity profiles have been shifted for clarity. The PRPP synthase activity of the wild-type strain is shifted by 2 nmol/(min min $^{-1}$), that of the $\Delta prs2$ strain by 0.5 nmol/(min min $^{-1}$), that of the $\Delta prs3$ strain by 1.0 nmol/(min min $^{-1}$), and that of the $\Delta prs4$ strain by 1.5 nmol/(min min $^{-1}$). (B) Ion exchange chromatography. One milliliter of crude extract (1.07 μ mol min $^{-1}$ of PRPP synthase activity) of wild-type strain YN94-2 was applied to the column. Red line, PRPP synthase activity. The blue line shows the calculated sodium chloride concentration measured as conductivity by the equipment.

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